Response to reviews of manuscript "Trade-offs between water loss and carbon gain in a subtropical primary forest on Karst soils in China" bg-2018-44

Response to reviewer#2

Dear Reviewer,

We would like to thank you for the thoughtful and valuable comments and suggestions on our manuscript entitled "Trade-offs between water loss and carbon gain in a subtropical primary forest on Karst soils in China" (bg-2018-44). We have carefully revised our manuscript to take account of your comments and suggestions. Please find below our responses (upright Roman) to comments (original queries in Italic). Meanwhile, we have rephrased our manuscript title as "The strategies of water-carbon regulation of plants in a subtropical primary forest on Karst soils in China". The line numbers mentioned here refer to our original manuscript. The changed figures and tables are presented in the Appendix (listed at the end of the "Response to reviewer").

General comments:

(1) The author use "Trade-offs between water loss and carbon gain" in the title, however, the whole-text actually talk about the limitation of different components on A and iWUE.

Response: Thanks a lot for your comment. We response to this comment from two aspects. One on hand, we have rephrased our manuscript title as "The strategies of water-carbon regulation of plants in a subtropical primary forest on Karst soils in China".

On the other hand, we have revised the Section "Discussion". Firstly, we have re-organized and revised Section "4.1 The role of g_m in CO_2 diffusion and V_{cmax}", and merged it with "4.2 Co-variation in g_s, g_m and V_{cmax} in regulating A ". Such as, we have moved two paragraphs "Three methods are most commonly used for g_m estimation. ..... All of these methods are based on gas exchange measurements (Pons

et al., 2009), and some common assumptions (Warren, 2006). Thus, the accuracy of each method is largely unknown (Warren, 2006) (Lines 288-295).

The g_m was estimated by the 'curve-fitting' method in this study. ... We confirmed that the calculated C_c and the initial slope of A-C_c curves were positive, suggesting that the measured g_m was reliable (Warren, 2006). (Lines 297-304)" to Section "Methods and Materials".

We have deleted two paragraphs "Large uncertainties can be introduced by ignoring g_m. .... $\Delta^{13}C\_g_m$ represented the carbon isotope discrimination when g_m was finite, and $\Delta^{13}C\_g_s$ represented the carbon isotope discrimination when g_m was infinite (Lines 319-328).

On the other hand, ignoring g_m would underestimate V_{cmax} up to 75% (Sun et al., 2014). .... Furthermore, the leaf barrier to CO_2 caused by g_m has not been represented in the global carbon cycles, leading to an overestimation of CO_2 supply for carboxylation and an underestimation of the response of photosynthesis to atmospheric CO_2 (Sun et al., 2014) (Lines 330-337).".

We have revised the paragraph "Large variability in g_m has been shown both between and within species with different leaf forms and habits (Gago et al., 2014; Flexas et al., 2016). .... Hence, the wide variability of g_m between different species and life forms in the same ecosystem seems to be related to the diversity in leaf anatomical traits. (Lines 306-317)" to "The importance of g_m in constraining A was variable, and depended on leaf structural traits, only LMA, LT, and LD were analyzed in this study. Large variability in g_m has been shown both between and within species with different life forms and habits (Gago et al., 2014; Flexas et al., 2016). Variability in g_m in this study is similar to that in global datasets (Gago et al., 2014; Flexas et al., 2016). There was no significantly difference among life forms ($P>0.05$). Previous studies have confirmed that LMA (Tomas et al., 2013), thickness of leaf cell wall (Peguero-Pina et al., 2017b), liquid phase of mesophyll (Veromann-Jurgenson et al., 2017), cell wall thickness of mesophyll (Terashima et al., 2011;Tosens et al., 2016), and surface area

of mesophyll and chloroplast exposed to intercellular space (Veromann-Jurgenson et al., 2017) were the main limitations for g_m. The wide variability of g_m between different species and life forms in the same ecosystem seems to be related to the diversity of leaf anatomical traits.". And we have merged this paragraph with "4.2 Co-variation in g_s, g_m and V_{cmax} in regulating A ".

Secondly, we revised the title of Section "4.2 Co-variation in g_s, g_m and V_{cmax} in regulating A" as "4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A ". And we have re-analyzed our data, and revised the paragraph "The A was constrained by g_s, g_m, and V_{cmax} acting together, however, variability in the relative contribution of these three factors depended on species and habitats (Tosens et al., 2016; Galmes et al., 2017; Peguero-Pina et al., 2017a; Veromann-Jurgenson et al., 2017)..... In addition, 20 of the 63 species were mainly limited by V_{cmax} (l_b>0.4, with the largest value of 0.68). (Lines 340-351) " to "A was constrained by g_s, g_m, and V_{cmax} acting together, however, variability in the relative contribution of these three factors depended on species and habitats (Tosens et al., 2016; Galmes et al., 2017; Peguero-Pina et al., 2017a; Veromann-Jurgenson et al., 2017). A was significantly correlated with g_s, g_m, and V_{cmax} (Fig.3a-c). g_s was positively related to g_m (Fig.S1c), while no relationship was found between the CO_2 diffusion conductance (g_s and g_m) and V_{cmax} (Fig. S2). The relative limitations of g_s, g_m, and V_{cmax} were separated by a quantitative limitation model (Jones, 1985; Grassi & Magnani, 2005). The results showed that l_s, l_m and l_b of 63 species varied in a large range (Fig. S3), indicating plants have a diverse strategies to co-ordinate the CO_2 diffusion (g_s and g_m) and V_{cmax} to maintain relative high A. The order of factors limitations to A was l_m> l_b >l_s (P<0.05) (Fig.S3). Furthermore, we tested the relationship between the relative limitations and the corresponding limitation factors. The results showed that l_s, l_m, and l_b were negatively associated with g_s, g_m, and V_{cmax}, respectively (Fig. 4). And the relationship was stronger for g_m-l_m (r^2=0.65) than V_{cmax}- l_b (r^2=0.27) and g_s- l_s (r^2=0.19).

g_s was better correlated with A, while the results showed that A was more limited by g_m. That could be explained by two possible reasons. Firstly, compare to the linear

relationship between A and g_s, a nonlinear trend has been found between A and g_m when g_m>0.4 (Fig. 3a, b). Secondly, leaf structure plays an important role in regulating g_m and V_{cmax}, consequently, in determining A (Veromann-Jurgenson et al., 2017). Negative relationships between A/LMA and LT (r^2=0.16, p=0.002), and A/LMA and LT (r^2=0.3, p<0.001) have been observed (Fig. S4c,d), while A was not correlated to LT and LD (Fig. S4a,b)."

We have tested the difference of LMA, leaf thickness (LT) and leaf density (LD) among life forms, no significantly different have been found. And then we tested the roles of leaf structure (LT and LD) on A, g_m, and V_{cmax}. The results showed that leaf structure plays important role in regulating A, g_m, and V_{cmax}. Consequently, we revised discussions about the carbon fixation (A) strategies of plants (Lines 353-406) as "The importance of g_m in constraining A was variable, and depended on leaf structural traits, only LMA, LT, and LD were analyzed in this study. Large variability in g_m has been shown both between and within species with different life forms and habits (Gago et al., 2014; Flexas et al., 2016). Variability in g_m in this study is similar to that in global datasets (Gago et al., 2014; Flexas et al., 2016). There was no significantly difference among life forms (P>0.05). Previous studies have confirmed that LMA (Tomas et al., 2013), thickness of leaf cell wall (Peguero-Pina et al., 2017b), liquid phase of mesophyll (Veromann-Jurgenson et al., 2017), cell wall thickness of mesophyll (Terashima et al., 2011;Tosens et al., 2016), and surface area of mesophyll and chloroplast exposed to intercellular space (Veromann-Jurgenson et al., 2017) were the main limitations for g_m. The wide variability of g_m between different species and life forms in the same ecosystem seems to be related to the diversity of leaf anatomical traits.

No significant difference of LMA, LT, and LD was found among life forms (P<0.05). The negative correlation of g_m (Terashima et al., 2005) or g_m/LMA (Niinemets et al., 2009; Veromann-Jurgenson et al., 2017) with LMA have been reported. In this study, there was a significant relationship between g_m/LMA with LMA (P<0.01), however, no relationship was found between g_m with LMA. g_m/LMA was significantly negative

related to LD ($p<0.01$) (Fig. S5c), and weak negative related to LT ($p=0.06$) (Fig. S5d), demonstrating that the negative role of cell wall thickness on g_m (Terashima et al., 2006; Niinemets et al., 2009). The strong investment in supportive structures was the main reason for the limitation of g_m on A (Veromann-Jurgenson et al., 2017). However, it is still unknown how leaf anatomical traits affect g_m and A, and this should be further explored.

g_s is responsible for CO_2 exchange between atmosphere and leaf, and regulate the CO_2 fixation (A) and water loss (Lawsonand Blatt, 2014). The variability of g_s was controlled by stomatal anatomy, i.e. stomata density and size, and mesophyll demands for CO_2 (Lawsonand Blatt, 2014). However, the stomatal anatomy was not analyzed in this study. We only focused on how the relationship between g_s and g_m regulate A. Positive relationship between g_s and g_m has been observed (Flexas et al., 2013). For example. the restricted CO_2 diffusion from the ambient air to chloroplast is the main reason for a decreased A under water stress conditions due to both the stomatal and mesophyll limitations (Olsovska et al., 2016). g_s was significantly positive related to g_m for 63 species ($P<0.001$, Fig. S1) in this study, and no difference of the slopes of regression lines between g_s and g_m was found among life forms, demonstrating that A was regulated by the co-variation of g_s and g_m. However, the variability of g_m and l_m was larger than g_s and l_s, respectively (Fig.1 and Fig.S3).

The wide variation range of l_b (0.11-0.68) highlighted the importance role of V_{cmax} in regulating A. V_{cmax} was used to represent the CO_2 demand in photosynthetic process in this study. The relative contribution of V_{cmax} to A not only depends on C_a-C_c, but also on leaf nutrient levels. Positive relationship was found between C_a-C_c and V_{cmax} (Fig. 1d). And the V_{cmax}/LMA was co-regulated by leaf N, P and Mg content (Jing et al. 2018). In addition, V_{cmax}/LMA was negatively related to LT ($p<0.05$) (Fig. S6c) and LD ($p<0.05$) (Fig. S6d), while V_{cmax} was not correlated to LT and LD (Fig. S6a,b), demonstrating that leaf structure plays an important role in regulating V_{cmax}.

The trade-off between CO_2 supply (g_s and g_m) and demand (carboxylation capacity of Rubisco) can help maintain relative high A (Galmes et al., 2017; Saez et al., 2017). In this study, we used V_{cmax} as a proxy for the carboxylation capacity of Rubisco, and the

normalized V_{cmax} by A (V=V_{cmax}/A) was significantly negatively correlated with the normalized g_t by A (G_t =g_t/A) (P<0.001) (Fig. 2c), indicating that the trade-off between CO_2 supply and demand also existed among different species in the same ecosystems. For genus *Limonium* (flowering plants) (Galmes et al., 2017), g_t was significantly positively related to Rubisco carboxylase specific activity, and significantly negatively related to Rubisco specificity factor to CO_2. In case of Antarctic vascular (Saez et al., 2017) and Mediterranean plants (Flexas et al., 2014), A was mainly limited by low g_m, but it could be partially counterbalanced by a highly efficient Rubisco through high specificity for CO_2. This highlights the importance of the trade-off between CO_2 supply and demand in plant adaptation to Karst environment. However, it is still unknown how leaf anatomical traits affect g_m, V_{cmax} and A, and this should be further explored. ”

Thirdly, we have revised the title of Section “4.3 Co-variation in g_s, g_m and V_{cmax} in regulating iWUE” as “4.2 Co-variation in g_s, g_m and V_{cmax} in regulating iWUE ”. To emphasize the diverse carbon-water regulation strategies of plants in Karst CZs, and highlighted the role of trade-off between carbon gain and water loss, we have revised the paragraph “Compared with the global dataset under well-watered conditions (19.27-171.88 μmol CO_2 mol^{-1} H_2O) (Flexas et al., 2016), the iWUE (29.52-88.92 μmol CO_2 mol^{-1} H_2O) in this study was somewhat lower in this study....The average iWUE of 12 vines and 13 trees in the Karst tropical primary forest was 41.23±13.21 μmol CO_2 mol^{-1} H_2O (Chen et al., 2015), while that of 6 evergreen and 6 deciduous trees was 66.7±4.9 and 49.7±2.0 μmol CO_2 mol^{-1} H_2O, respectively (Fu et al., 2012). (Lines 409-422)” to “Compared with the global dataset under well-watered conditions (19.27-171.88 μmol CO_2 mol^{-1} H_2O) (Flexas et al., 2016), iWUE (52.85±13.08 μmol CO_2 mol^{-1} H_2O) was somewhat lower in this study. iWUE varied from 29.53 to 88.91 μmol CO_2 mol^{-1} H_2O, and the variability of iWUE was larger than in the Karst tropical primary forest (Fu et al., 2012; Chen et al., 2015). The average iWUE of 12 Vines and 13 Trees in the Karst tropical primary forest was 41.23±13.21 μmol CO_2 mol^{-1} H_2O (Chen et al., 2015), while that of 6 evergreen and 6 deciduous Trees was

66.7±4.9 and 49.7±2.0 μmol CO_2 mol^{-1} H_2O, respectively (Fu et al., 2012). The results demonstrated that Karst plants use a diverse strategies of carbon-water regulation to adapt to the harsh Karst environment.

The trade-off between carbon gain and water loss is one of important strategies of carbon-water regulation of plants, and was exist among species and life forms (Prentice et al., 2014). Prentice et al. (2014) studied the trade-off between carbon gain and water loss of woody species in contrasting climates, and found that species in hot and wet regions tend to lose more water in order to fix more carbon (high g_s/A, low V_{cmax_Ci}/A), and vice versa. Although Karst soils cannot contain enough water for plant growth, the trade-off between carbon gain and water loss (high g_s/A and low V_{cmax_Ci}/A) were similar to the shown for plants growing in hot and wet regions (Prentice et al., 2014). ”

(2) In the method section: The species covered wide range of functional groups, including 6 life forms. What the criteria of the species selection? Because the leaf habit (evergreen or deciduous), the shade or light-demanding behaviors also will affect the strategy of plant carbon-water regulation. For example, does fern grow in the canopy or understory, how you can put them together when analyze the data? More important, the main objective of this paper was to determine and distinguish the limitations of CO2 diffusion and Vcmax on A and iWUE in different life forms Karst forest, however, you combine all species together for most analysis, actually we donot know what's the difference between different life forms in Figs 1-4, 6,7. I Believe most land plant will behave in similar way to adapt to the environmental factor no matter where they grow, the interesting things is to what extent by different plants. For example, Based on Fig 5, we could not see any difference among the groups. So, I suggest the author should separate into 6 groups to see the differences of regression lines among groups for all the figures, and compare the difference among the life forms using proper statistical method.

Response: Thank you for your comments and suggestions. We response to revised the manuscript from three aspects according to your comments and suggestions. Firstly,

we have added our criteria of the species selection in Section “2.2 Leaf gas-exchange measurements” “In July and August 2016, 63 species (Table S1) were selected for measurements of the *A* and CO_2 response curves. The species sampled were selected according to their abundance in the study site. They are the main component of this forest, including 55 woody species (46 deciduous and 10 evergreen species) and 5 herb species. To distinguish the strategies of water-carbon regulation of plants among different life forms, those species were grouped into 6 life forms, including (1) Tree (n=29), (2) Tree/Shrub (n=11), (3) Shrub (n=11), (4) Grass (n=11), (5) Vine (n=5), and (6) Fern (n=3). “Tree/Shrub” is a kind of low wood plant between Tree and Shrub. Fern grow in understory. Vine climb up to the shrub canopy to get light. ” We have added how were the leaves collected “Branches exposed to the sun were excised from the upper part of the crown (Trees, Tree/Shrubs, Shrubs and Vines) or aboveground portion (Grasses, Ferns), and immediately re-cut under water to maintain xylem water continuity. ”.

Secondly, we have re-analyzed our data either as a whole group (six life forms combined) or by individual life forms, and the difference between different life forms was tested using the standardized major axis (SMA) regression fits. The results showed that no significantly difference between life forms. Thus six life forms were grouped together to analyze the strategy of water-carbon regulation of plants in the whole text. The statistical method and results have been added in Section “2.5 Statistical analysis” “Data were analyzed either as a whole group (six life forms combined) or by individual life forms. The bivariate linear regressions of leaf gas exchange parameters were performed using the standardized major axis (SMA) regression fits, and all of the data were made on $\log_e$-transformed data (Table S2).

To test for the differences among life forms, SMA regression fits were used to compare the slope of regression lines which significant relationships had already been obtained. Note that Grass, Vine and Fern were not considered due to the small sample size. A similar trend was obtained, and no significant difference was found between life forms although significant relationships were not obtained for some bivariate

linear regressions. Accordingly, six life forms were grouped together to analyze the strategy of water-carbon regulation of plants in the whole text.

The difference of relative limitation of g_s, g_m and V_{cmax} to A for life forms or as a whole group were performed using one-way ANOVA and Duncan multiple comparison. The probability of significance was defined at $p< 0.05$. ”

Thirdly, all of data of six life forms were separately presented in Figure 1-4, 6,7 and Figure S1,S2, S4-S6 (See Appendix). Only the regression line for 63 species were presented in figures.

(3) lines 139-140, because the A-Ci curve is the key data of this paper, author should describe in detail how this measurement was done rather than just cite other submitted papers. For example, you should introduce the height of your targeted individuals? how you can measure the sun-exposed leaf for canopy trees and climbing plants: : :.?did you measure in situ or cut down, if the latter, for A-Ci curve you normally need ca. 30 min, how you can avoid the effects of cutting on stomatal conductance because some species are very sensitive, do you have some information on the gs sensitivity for those species ïj §: : :..

Response: Thank you for your suggestions. In response, we have added more details about leaf sampling and measurements in Section “Materials and Methods”. Such as, we have added the method of how were the leaves collected and prepared before CO_2 response curves measurements “Details of leaf sampling and measurements of the CO_2 response curve were briefly described as follows. Branches exposed to the sun were excised from the upper part of the crown (Trees, Tree/Shrubs, Shrubs and Vines) or above the ground (Grasses, Ferns), and immediately re-cut under water to maintain xylem water continuity. Back into the laboratory, branches were kept at 25^{o}C for 30 min. Fully-expanded and mature leaves were induced for 30 minutes at a saturating light density (1500 μmol m^{-2} s^{-1}). CO_2 response curves measurements were performed when A and g_s was stable. Three leaves per species were collected and measured. A

total of 189 leaves were collected from adult individuals of 63 species." However, the height of targeted individuals did not measured.

We have described the method and conditions of CO_2 response curves measurements in more detail as: "The CO_2 response curves were measured with 11 CO_2 concentration gradients in chamber following the procedural guidelines described by Longand Bernacchi (2003). The photosynthetic photon flux density was 1500 μmol m^{-2} s^{-1}. The leaf temperature was 25 ℃, controlled by the block temperature. The humidity in the leaf chamber was maintained at ambient condition. Leaf area, thickness (LT) and dry mass were measured after the CO_2 response measurements. Leaf mass per area (LMA) was calculated by dividing the corresponding dry mass by leaf area. And leaf density (LD) was calculated by dividing the corresponding LMA by LT. More details were described in Wang et al. (2018)."

Specific comments:

(4) Line 267-269: There is no statistic tests of the differences of the results in figure 5, so it is not proper to give the statements in line 309-310. Figure 5 can't give any information that is about LMA. Please use data to demonstrate the relationship between LMA and other parameters instead of qualitative description.

Response: Thank you for your comments and suggestions. We response to the comments and suggestions from two aspects. Firstly, we have analyzed the data of figure 5 using statistical method, and revised the corresponding Sections. Such as, we have added statistical method used to test the difference of the results in figure 5 in Section "2.5 Statistical analysis" "The difference of relative limitation of g_s, g_m and V_{cmax} to A for life forms or as a whole group were performed using one-way ANOVA and Duncan multiple comparison. The probability of significance was defined at $p < 0.05$." .

We have drew figure 5 and revised the Section "3.2 Contribution of g_s, g_m and V_{cmax} to A" as "The variation in A was attributed to variation in g_s, g_m, g_t, and V_{cmax}. A was positively correlated with g_s (Fig. 3a), g_m (Fig. 3b), and V_{cmax} (Fig. 3c). We used the

quantitative limitation model (Eqs. (9), (10) and (11)) to separate g_s (l_s), g_m (l_m), and V_{cmax} (l_b) limitations to *A*. l_s, l_m, and l_b were negatively associated with g_s, g_m, and V_{cmax}, respectively (Fig. 4). The contributions by g_s, g_m, and V_{cmax} to limiting *A* were different for each species (Fig. S3). l_s varied 2.6-fold (from 0.17 to 0.45), l_m varied 10.5-fold (from 0.05 to 0.55), and l_b varied 6.2-fold (from 0.11 to 0.68) across species. Overall, l_m (0.38±0.12) was significantly larger than l_b (0.34±0.14), and l_s (0.28±0.07) ($P<0.05$).

To further understand how *A* was limited by g_s, g_m, and V_{cmax} among life forms, we grouped the 63 species into 6 life forms: Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern. The results showed that there was no significantly difference between l_s, l_m and l_b for Trees and Tree/shrubs. l_m of Shrubs and Grasses was significantly higher than that of l_s and l_b ($P<0.05$). l_m of Vines and Ferns was significantly higher than that of l_s ($P<0.05$) (Fig. 5). ".

We have revised the Section "4.1 Co-variation in g_s, g_m and V_{cmax} in regulating *A* ". Please also see the response to reviewer #1.

Secondly, we have tested the difference of LMA across life forms using one-way ANOVA and Duncan multiple comparison. The results showed that no difference of LMA was found among life forms. Consequently, lines 309-310 have been removed. We have tested the role of leaf structure (leaf thickness (LT) and leaf density (LD)) in *A*, g_m and V_{cmax}, and rephrased the Section "4.1 Co-variation in g_s, g_m and V_{cmax} in regulating *A* ". Please also see the response to reviewer #1.

(5) Line 372: Species with low LMA may have thick cell walls in mesophyll and chloroplast.

Response: Thank you for your suggestion. We have tested the difference of LMA across life forms using one-way ANOVA and Duncan multiple comparison. The results showed that no difference of LMA was found among life forms. Meanwhile, We have tested the role of leaf structure (leaf thickness (LT) and leaf density (LD)) in *A*, g_m and V_{cmax}. The results showed that leaf structure plays important role in

regulating g_m and V_{cmax}, consequently, in determining A. Consequently, we revised the corresponding section in "4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A " as "The importance of g_m in constraining A was variable, and depended on leaf structural traits, only LMA, LT, and LD were analyzed in this study. Large variability in g_m has been shown both between and within species with different life forms and habits (Gago et al., 2014; Flexas et al., 2016). Variability in g_m in this study is similar to that in global datasets (Gago et al., 2014; Flexas et al., 2016). There was no significantly difference among life forms ($P>0.05$). Previous studies have confirmed that LMA (Tomas et al., 2013), thickness of leaf cell wall (Peguero-Pina et al., 2017b), liquid phase of mesophyll (Veromann-Jurgenson et al., 2017), cell wall thickness of mesophyll (Terashima et al., 2011;Tosens et al., 2016), and surface area of mesophyll and chloroplast exposed to intercellular space (Veromann-Jurgenson et al., 2017) were the main limitations for g_m. The wide variability of g_m between different species and life forms in the same ecosystem seems to be related to the diversity of leaf anatomical traits.

No significant difference of LMA, LT, and LD was found among life forms ($P<0.05$). The negative correlation of g_m (Terashima et al., 2005) or g_m/LMA (Niinemets et al., 2009; Veromann-Jurgenson et al., 2017) with LMA have been reported. In this study, there was a significant relationship between g_m/LMA with LMA ($P<0.01$), however, no relationship was found between g_m with LMA. g_m/LMA was significantly negative related to LD ($p<0.01$) (Fig. S5c), and weak negative related to LT ($p=0.06$) (Fig. S5d), demonstrating that the negative role of cell wall thickness on g_m (Terashima et al., 2006; Niinemets et al., 2009). The strong investment in supportive structures was the main reason for the limitation of g_m on A (Veromann-Jurgenson et al., 2017). However, it is still unknown how leaf anatomical traits affect g_m and A, and this should be further explored. "

(6) Line 381-382: In your results, gs and gm are positively correlated, why did you conclude gm is a compensate for reductions in gs? Did you observe an increasing of gm when gs decreased.

Response: Thank you for your comment. We corrected this mistake, and we rephrased this paragraph as: "g_s is responsible for CO_2 exchange between atmosphere and leaf, and regulate the CO_2 fixation (A) and water loss (Lawsonand Blatt, 2014). The variability of g_s was controlled by stomatal anatomy, i.e. stomata density and size, and mesophyll demands for CO_2 (Lawsonand Blatt, 2014). However, the stomatal anatomy was not analyzed in this study. We only focused on how the relationship between g_s and g_m regulate A. Positive relationship between g_s and g_m has been observed (Flexas et al., 2013). For example. the restricted CO_2 diffusion from the ambient air to chloroplast is the main reason for a decreased A under water stress conditions due to both the stomatal and mesophyll limitations (Olsovska et al., 2016). g_s was significantly positive related to g_m for 63 species ($P<0.001$, Fig. S1) in this study, and no difference of the slopes of regression lines between g_s and g_m was found among life forms, demonstrating that A was regulated by the co-variation of g_s and g_m. However, the variability of g_m and l_m was larger than g_s and l_s, respectively (Fig.1 and Fig.S3)."

(7) Line 384-389: I don't think you have enough evidences to state "there was a trend of increasing lm with increasing leaf N:P", unless you add this part of research in your draft.

Response: Thank you for your comment. There was no significant statistical relationship between l_m and leaf N:P ($P=0.66$). We corrected this mistake, and rephrased this paragraph : " The wide variation range of l_b (0.11-0.68) highlighted the importance role of V_{cmax} in regulating A. V_{cmax} was used to represent the CO_2 demand in photosynthetic process in this study.The relative contribution of V_{cmax} to A not only depends on C_a-C_c, but also on leaf nutrient levels. Positive relationship was found between C_a-C_c and V_{cmax} (Fig. 1d). And the V_{cmax}/LMA was co-regulated by leaf N, P and Mg content (Jing et al. 2018). In addition, V_{cmax}/LMA was negatively related to LT ($p<0.05$) (Fig. S6c) and LD ($p<0.05$) (Fig. S6d), while V_{cmax} was not correlated to LT and LD (Fig. S6a,b), demonstrating that leaf structure plays an important role in regulating V_{cmax}."

(8) Awful sentences, Lines 39-35, should split into short sentences

Response: Rephrased as: "The results showed that (1) g_s and g_m varied about 7.6- and 34.5-fold, respectively, and g_s was positively related to g_m. The contribution of g_m to leaf CO_2 gradient was similar to that of g_s. The g_s/A, g_m/A and g_t/A was negative related to V_{cmax}/A. (2) the relative limitations of g_s (l_s), g_m (l_m) and V_{cmax} (l_b) to A for the whole group (combined 6 life forms) were significantly different from each other ($P<0.05$). l_m was the largest (0.38 ± 0.12), followed by l_b (0.34 ± 0.14) and l_s (0.28 ± 0.07). No significant difference was found between l_s, l_m, and l_b for Trees and Tree/shrubs, while l_m was the largest, followed by l_b and l_s for Shrubs, Grasses, Viens and Ferns ($P<0.05$). (3) iWUE varied about 3-fold (from 29.52 to 88.92 μmol CO_2 mol^{-1} H_2O) across all species, and was significantly correlated with g_s, V_{cmax}, g_m/g_s, and V_{cmax}/g_s."

Appendix

1 Figures

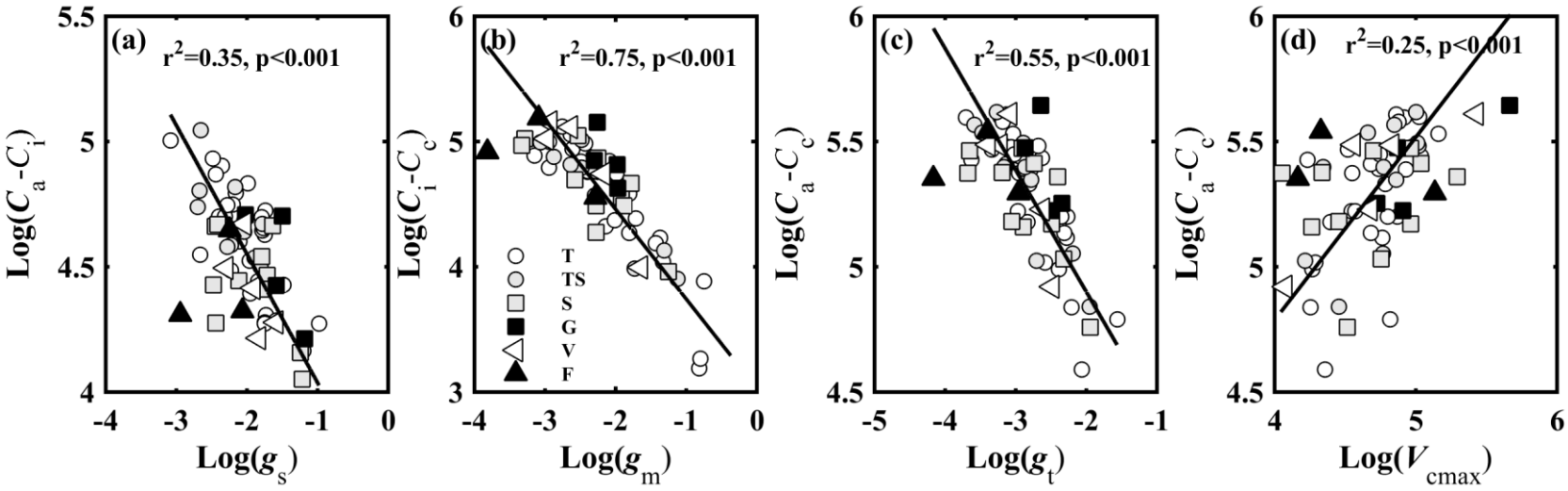

Figure 1. Relationships between (a) CO_2 gradient between ambient air and intercellular air space (C_a-C_i, μmol mol^{-1}) and stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}); (b) CO_2 gradient between intercellular air space and chloroplasts (C_i-C_c, μmol mol^{-1}) and mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}); (c) CO_2 concentration gradient between ambient air and chloroplasts (C_a-C_c, μmol mol^{-1}) and total conductance to CO_2 (g_t, mol CO_2 m^{-2} s^{-1}); and (d) C_a-C_c and the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

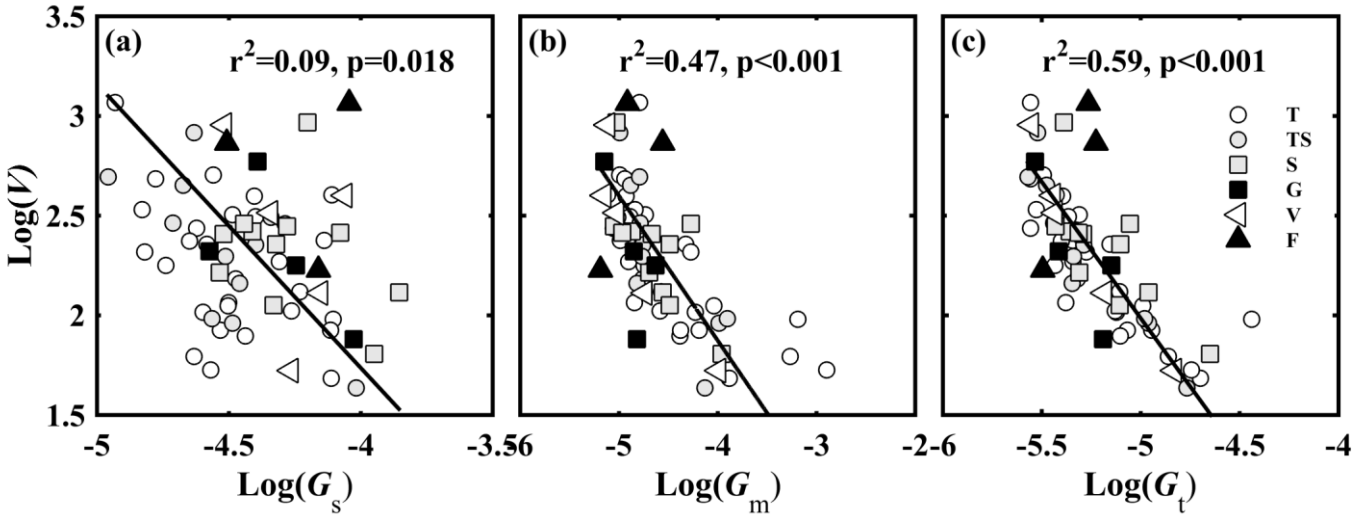

Figure 2. Relationships between (a) *V* and G_s; (b) *V* and G_m; and (c) *V* and G_t. *V* is the ratio of photosynthetic capacity (V_{cmax}) to light-saturated net photosynthesis (*A*, μmol CO_2 m^{-2} s^{-1}); G_s is the ratio of stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) to *A*; G_m is the ratio of mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) to *A*; G_t is the ratio of total conductance to CO_2 (g_t, mol CO_2 m^{-2} s^{-1}) to *A*. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

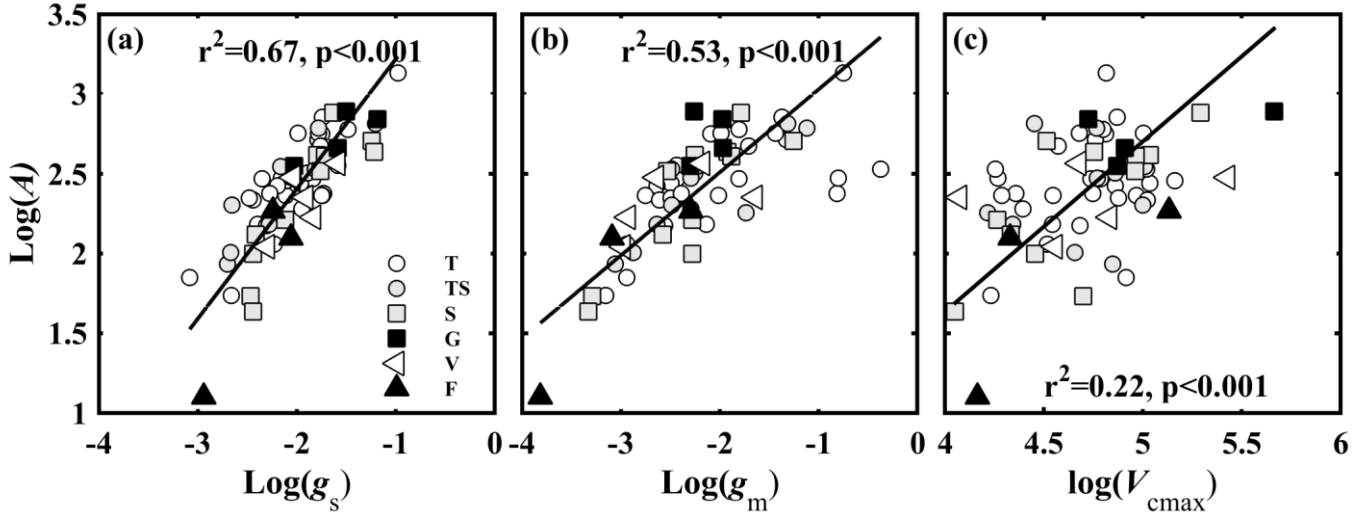

Figure 3. Relationships between light-saturated net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}) and (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}); and (c) the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

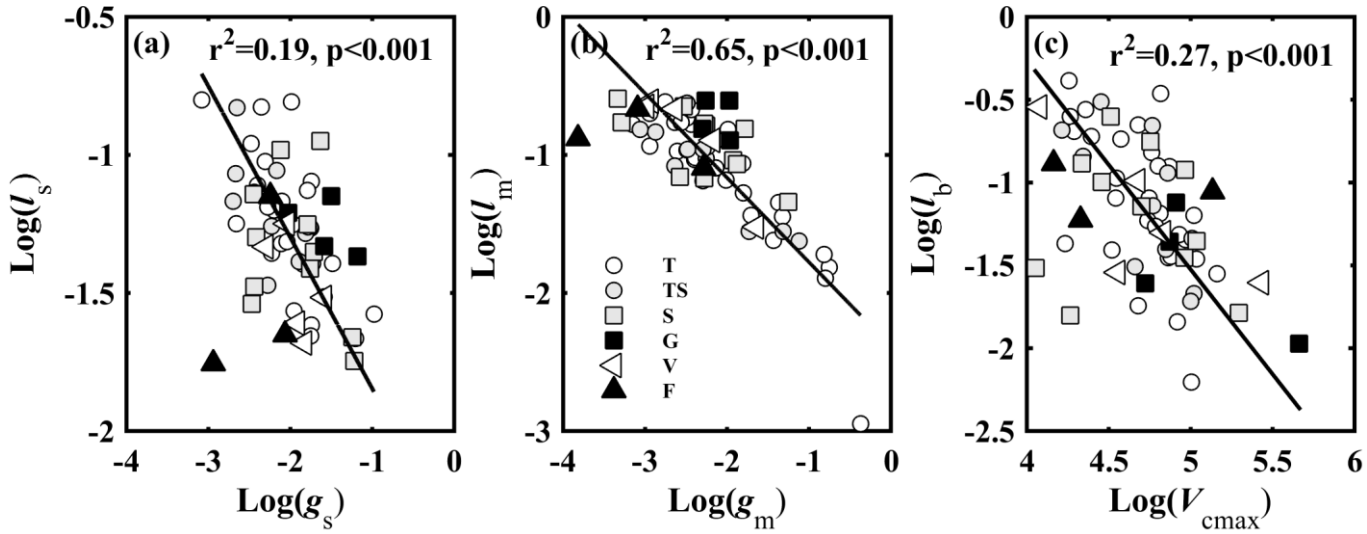

Figure 4. Relationships between (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) and l_s (g_s limitation on light-saturated net photosynthesis (A)); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and l_m (g_m limitation on A); and (c) the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}) and l_b (V_{cmax} limitation on A). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

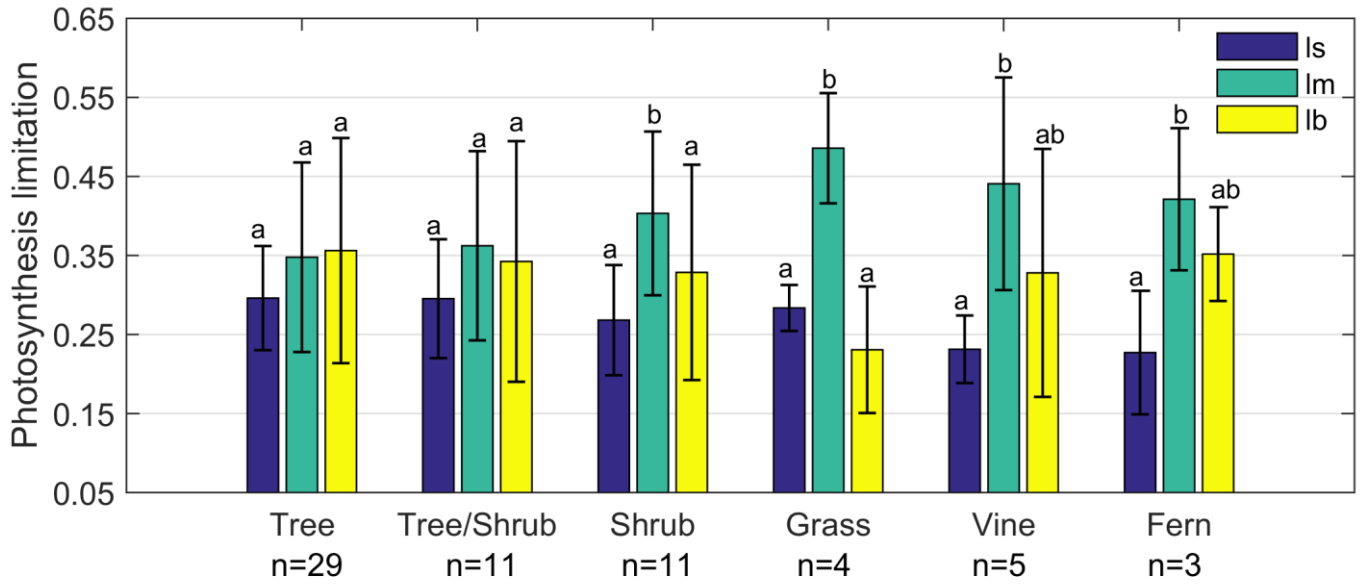

Figure 5. Limitation to light-saturated net photosynthesis (A) in six life forms by stomatal conductance to CO_2 (l_s), mesophyll conductance to CO_2 (l_m), and the maximum carboxylase activity of Rubisco (l_b). Error bars denominate standard deviation (1σ).

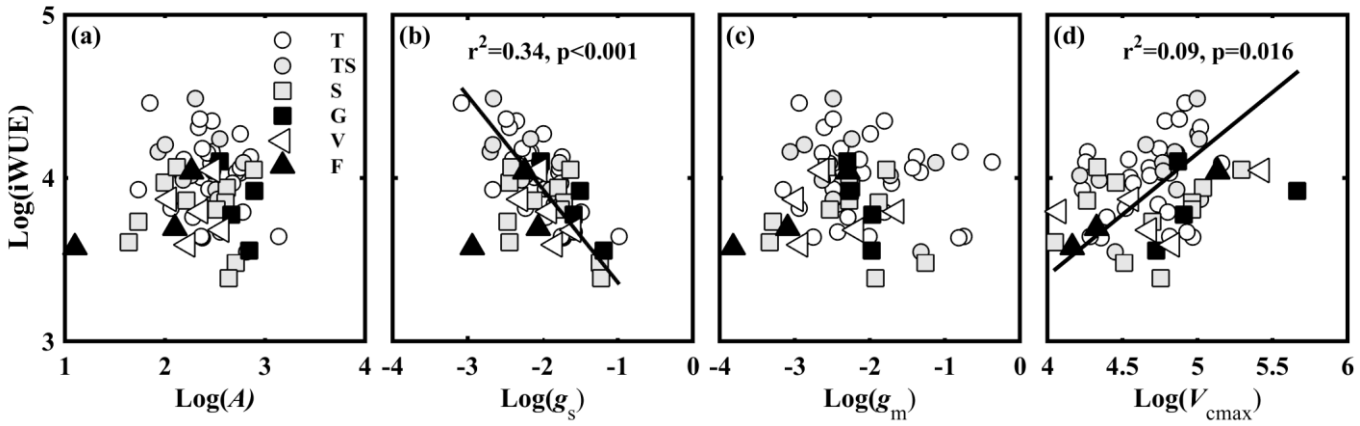

Figure 6. Relationships between the observed intrinsic water use efficiency (iWUE, μmol CO_2 mol^{-1} H_2O) and (a) light-saturated net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}); (b) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}); (c) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and (d) the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

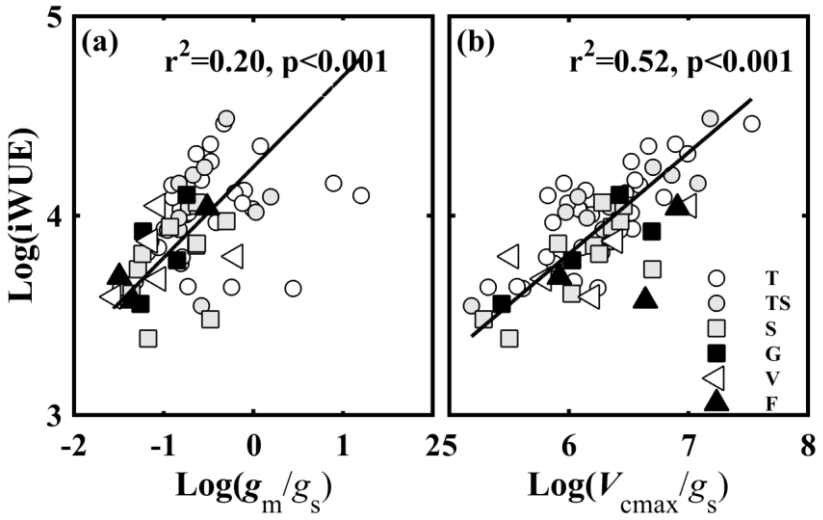

Figure 7. The relationships of the intrinsic water use efficiency (iWUE, μmol CO_2 mol^{-1} H_2O) and (a) the ratio of mesophyll conductance to CO_2 (g_m) to (g_s) (g_m/g_s) and (b) the ratio of the maximum carboxylase activity of Rubisco (V_{cmax}) to gs (V_{cmax}/g_s). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

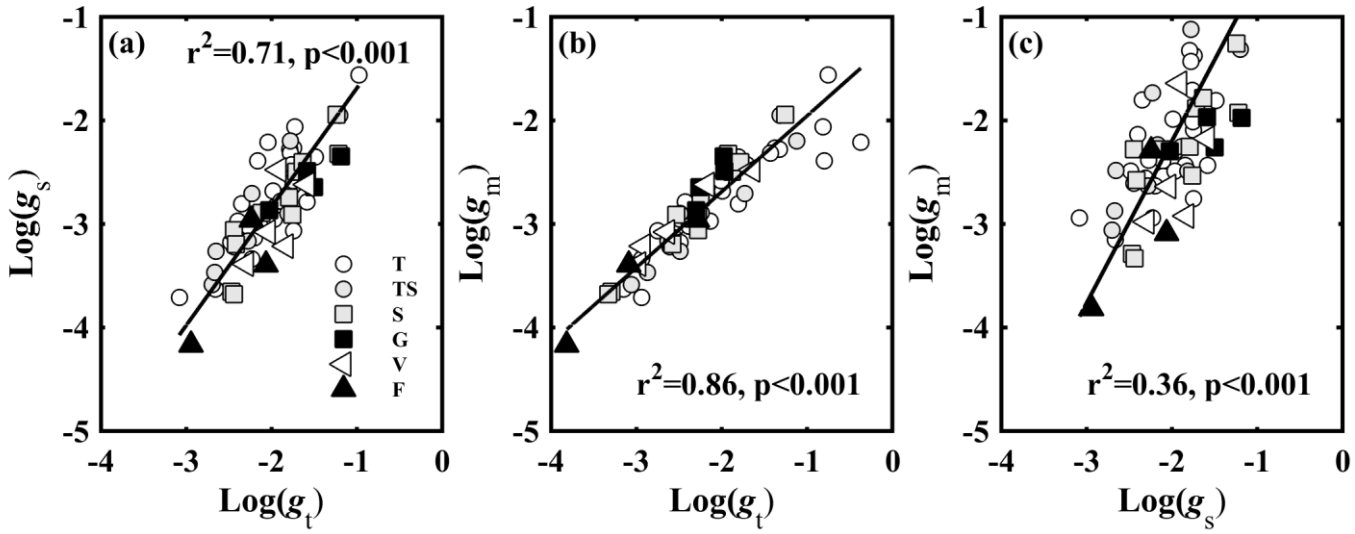

Figure S1 Relationship between (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) and total conductance to CO_2 (g_t, mol CO_2 m^{-2} s^{-1}); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and gt; and (c) g_s and g_m. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

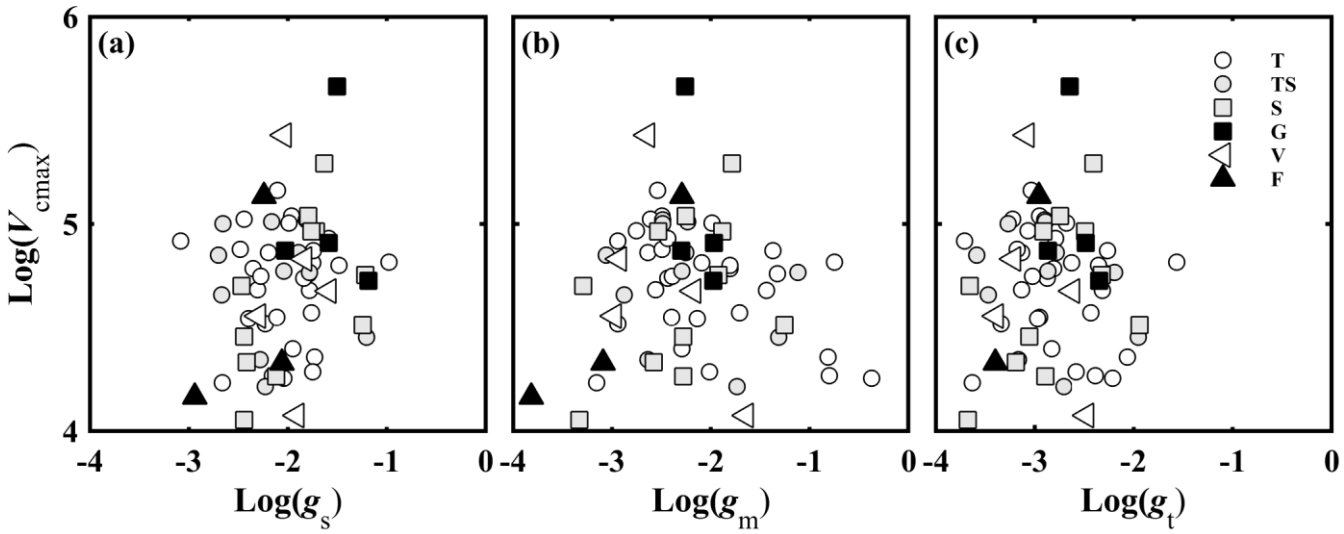

Figure S2 Relationship between (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) and the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and V_{cmax}; and (c) total conductance to CO_2 (g_t) and V_{cmax}. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

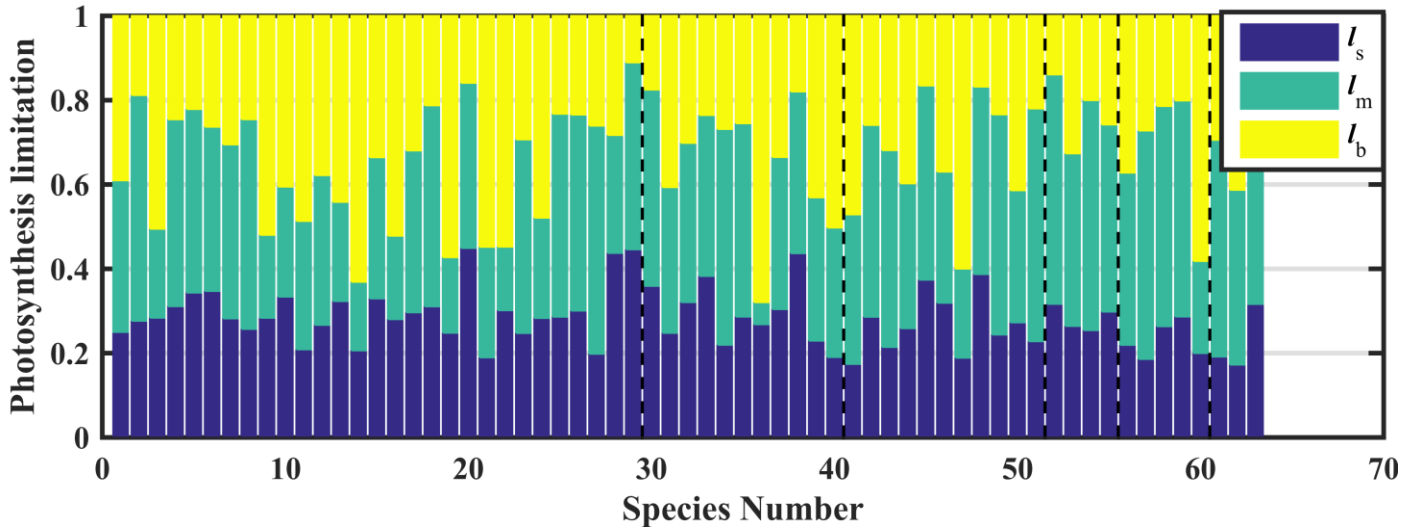

Figure S3 The limitation of (a) stomatal conductance to CO_2 (g_s) on photosynthesis rate (A) (l_s), (b) mesophyll conductance to CO_2 (g_m) on A (l_m) and (c) the maximum carboxylase activity of Rubisco (V_{cmax}) on A (l_b).

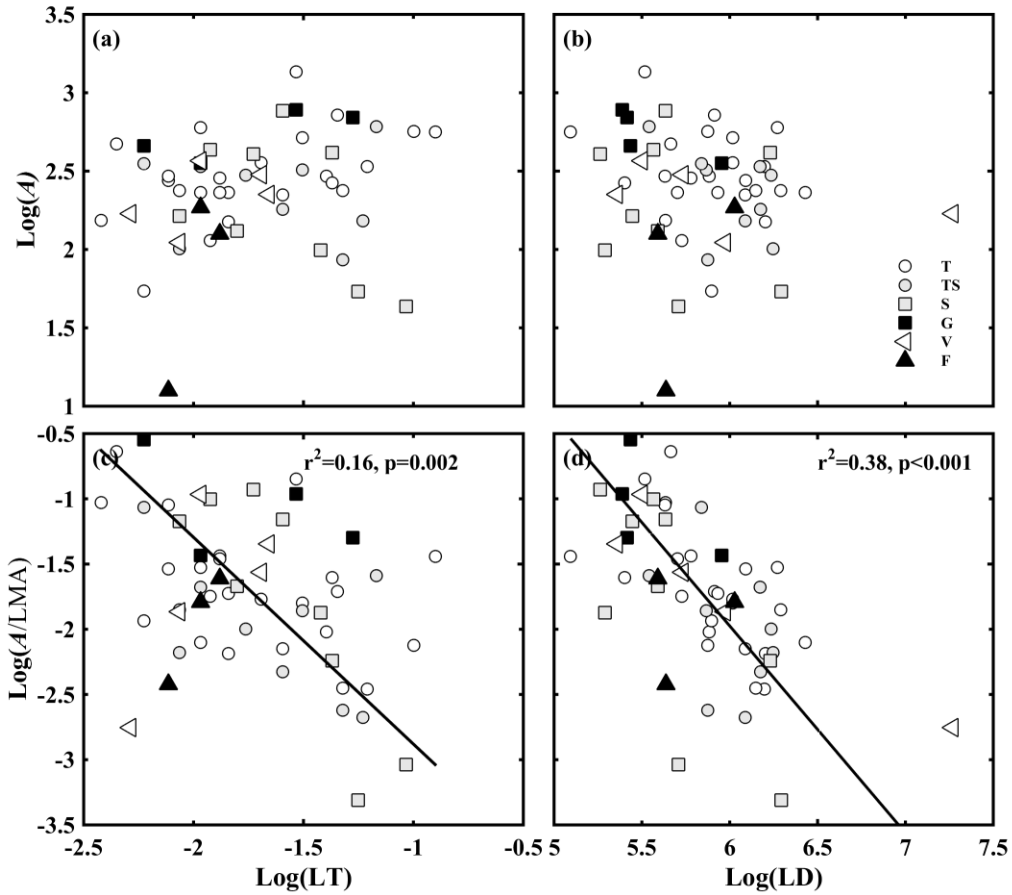

Figure S4 Relationship between (a) light-saturated net photosynthesis (*A*) and the leaf thickness (LT); (b) *A* and he leaf density (LD); (c) the ratio of *A* to leaf mass per area (LMA) (*A*/LMA); and (d) *A*/LMA and LD. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

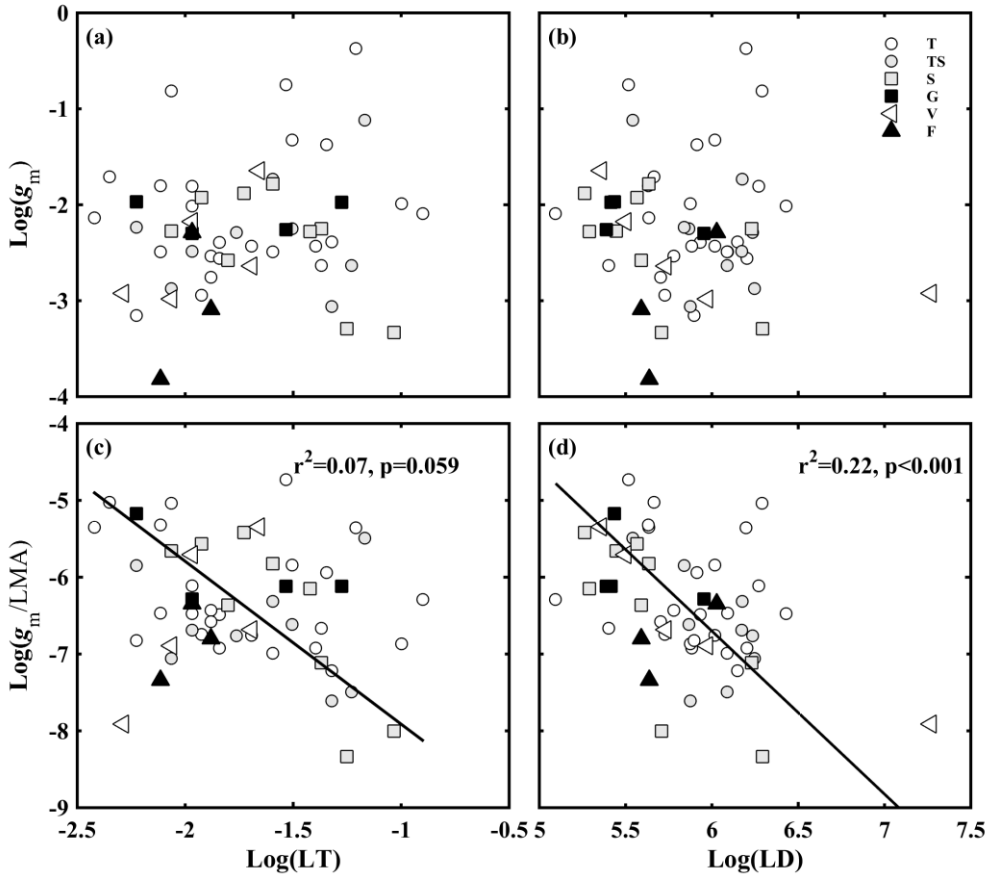

Figure S5 Relationship between (a) the mesophyll conductance to CO_2 (g_m) and the leaf thickness (LT); (b) g_m and he leaf density (LD); (c) the ratio of g_m to leaf mass per area (LMA) (g_m/LMA); and (d) g_m/LMA and LD. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

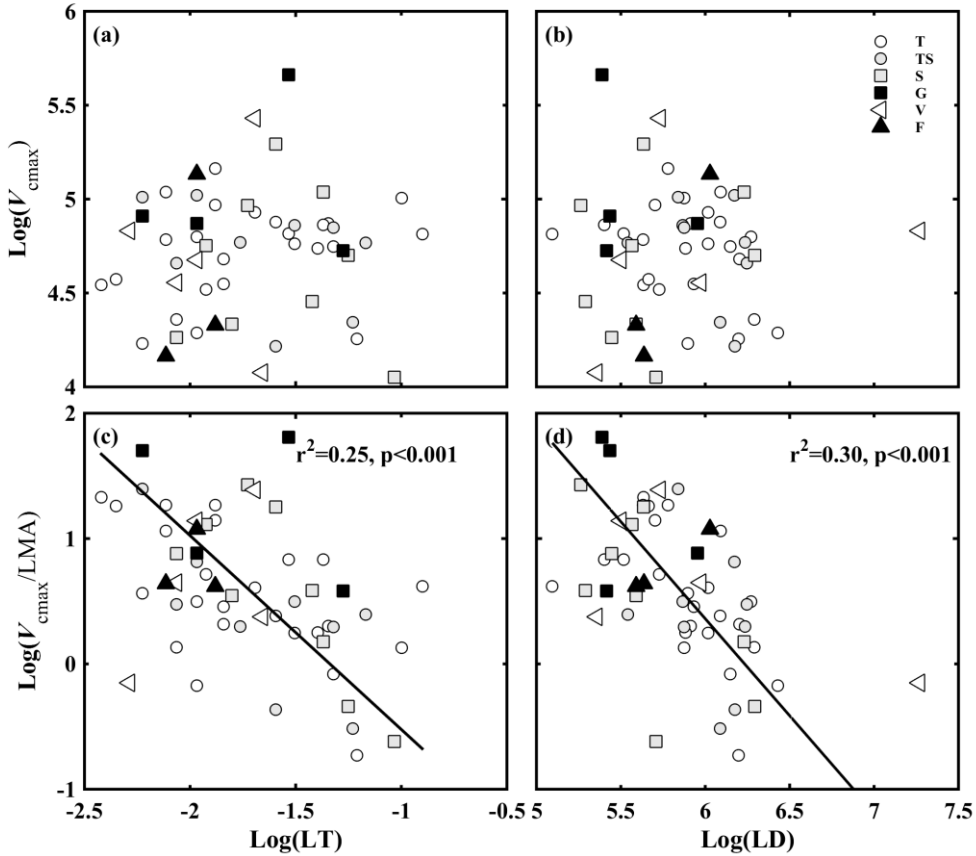

Figure S6 Relationship between (a) the maximum carboxylase activity of Rubisco (V_{cmax}) and the leaf thickness (LT); (b) V_{cmax} and he leaf density (LD); (c) the ratio of V_{cmax} to leaf mass per area (LMA) (V_{cmax}/LMA); and (d) V_{cmax}/LMA and LD. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

2 Tables

Table S1 Details information about the 63 species in the subtropical primary forest in Southwest China.

Species	Plant family		Life form	
Broussonetia papyifera (Linn.) L'Hert. ex Vent.	Moraceae	Tree	Deciduous	Woody
Machilus microcarpa Hemsl.	Lauraceae	Tree	Evergreen	Woody
Melia azedarach L.	Meliaceae	Tree	Deciduous	Woody
Populus ×canadensis Moench.	Salicaceae	Tree	Deciduous	Woody
Camptotheca acuminata Decne.	Nyssaceae	Tree	Deciduous	Woody
Cinnamomum bodinieri Levl.	Lauraceae	Tree	Evergreen	Woody
Catalpa ovata G. Don	Bignoniaceae	Tree	Deciduous	Woody
Toona sinensis (A. Juss.) Roem.	Meliaceae	Tree	Deciduous	Woody
Sapium sebiferum (Linn.) Roxb.	Euphorbiaceae	Tree	Deciduous	Woody
Cladrastis platycarpa (Maxim.) Makino	Leguminosae	Tree	Deciduous	Woody
Ulmus pumila L.	Ulmaceae	Tree	Deciduous	Woody
Ilex macrocarpa Oliv.	Aquifoliaceae	Tree	Deciduous	Woody
Vitex canescens Kurz	Verbenaceae	Tree	Deciduous	Woody
Eriobotrya japonica (Thunb.) Lindl.	Rosaceae	Tree	Evergreen	Woody
Morus alba L.	Moraceae	Tree	Deciduous	Woody
Prunus salicina Lindl.	Rosaceae	Tree	Deciduous	Woody
Eucommia ulmoides Oliver	Eucommiaceae	Tree	Deciduous	Woody
Platycarya strobilacea Sieb. et Zucc.	Juglandaceae	Tree	Deciduous	Woody
Kalopanax septemlobus (Thunb.) Koidz.	Araliaceae	Tree	Deciduous	Woody
Zanthoxylum armatum DC.	Rutaceae	Tree	Deciduous	Woody
Pyrus calleryana	Rosaceae	Tree	Deciduous	Woody
Amygdalus persica L. var.	Rosaceae	Tree	Deciduous	Woody

Euonymus meaackii Rupr.	Celastraceae	Tree	Deciduous	Woody
Zanthoxylum ovalifolium Wight	Rutaceae	Tree	Deciduous	Woody
Cerasus scopulorum (Koehne) Yu et Li	Rosaceae	Tree	Deciduous	Woody
Carpinus pubescens Burk.	Betulaceae	Tree	Deciduous	Woody
Lithocarpus confinis Huang	Fagaceae	Tree	Evergreen	Woody
Celtis sinensis Pers.	Ulmaceae	Tree	Deciduous	Woody
Diospyros kaki Thunb. var. silvestris Makino	Ebenaceae	Tree	Deciduous	Woody
Ligustrum lucidum Ait.	Oleaceae	Tree/Shrub	Deciduous	Woody
Rhamnus leptophylla Schneid.	Rhamnaceae	Tree/Shrub	Deciduous	Woody
Lindera communis Hemsl.	Lauraceae	Tree/Shrub	Evergreen	Woody
Itea yunnanensis Franch	Saxifragaceae	Tree/Shrub	Evergreen	Woody
Pittosporum brevicalyx (Oliv.) Gagnep	Pittosporaceae	Tree/Shrub	Evergreen	Woody
Litsea rubescens Lec.	Lauraceae	Tree/Shrub	Deciduous	Woody
Rhus chinensis Mill.	Anacardiaceae	Tree/Shrub	Deciduous	Woody
Alangium chinense (Lour.) Harms	Alangiaceae	Tree/Shrub	Deciduous	Woody
Evodia rutaecarpa (Juss.) Benth.	Rutaceae	Tree/Shrub	Deciduous	Woody
Machilus cavaleriei Levl.	Lauraceae	Tree/Shrub	Evergreen	Woody
Debregeasia longifolia (Burm. f.) Wedd.	Urticaceae	Tree/Shrub	Deciduous	Woody
Ziziphus jujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chow	Rhamnaceae	Shrub	Deciduous	Woody
Rubus inopertus (Diels) Focke	Rosaceae	Shrub	Deciduous	Woody
Coriaria nepalensis Wall.	Coriariaceae	Shrub	Deciduous	Woody
Celastrus orbiculatus Thunb.	Celastraceae	Shrub	Deciduous	Woody
Wikstroemia scytophylla Diels	Thymelaeaceae	Shrub	Deciduous	Woody
Viburnum foetidum Wall. var. ceanothoides (C. H. Wright) Hand.-Mazz.	Caprifoliaceae	Shrub	Deciduous	Woody
Hedera nepalensis K. Koch var. sinensis (Tobl.) Rehd.	Araliaceae	Shrub	Deciduous	Woody
Rubus parvifolius L.	Rosaceae	Shrub	Deciduous	Woody

Rosa roxbunghii	Rosaceae	Shrub	Deciduous	Woody
Mallotus repandus (Willd.) Muell. Arg.	Euphorbiaceae	Shrub	Deciduous	Woody
Mahonia bealei (Fort.) Carr.	Berberidaceae	Shrub	Evergreen	Woody
Fallopia multiflora (Thunb.) Harald.	Polygonaceae	Grass		Herb
Conyza canadensis (L.) Cronq.	Compositae	Grass		Herb
Ipomoea batatas (L.) Lam.	Convolvulaceae	Grass		Herb
Senecio scandens Buch.-Ham. ex D. Don	Compositae	Grass		Herb
Vitis piasezkii Maxim.	Vitaceae	Vien	Deciduous	Woody
Clematis urophylla Franch.	Ranunculaceae	Vien	Deciduous	Woody
Bauhinia glauca (Wall. ex Benth.) Benth.	Leguminosae	Vien	Evergreen	Woody
Caesalpinia decapetala (Roth) Alston	Leguminosae	Vien	Deciduous	Woody
Paederia scandens (Lour.) Merr.	Rubiaceae	Vien		Herb
Cyclosorus parasiticus (L.) Farwell.	Thelypteridaceae	Fern		
Cyrtomium fortunei J. Sm.	Dryopteridaceae	Fern		
Pteris vittata L.	Pteridaceae	Fern		

Table S2 Coefficients of determination of linear regressions of fig. 1-4 and fig.6-7.

		Fig.1		Fig.2		Fig.3		Fig.4		Fig.6		Fig.7	
Subgraph	Life form	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P
a	Total	0.35	0.000	0.09	0.018	0.67	0.000	0.19	0.000	0.00	0.922	0.20	0.000
	Tree	0.49	0.000	0.14	0.048	0.67	0.000	0.42	0.000	0.03	0.401	0.11	0.083
	Tree/Shrub	0.70	0.001	0.49	0.016	0.79	0.000	0.57	0.007	0.24	0.126	0.07	0.438
	Shrub	0.29	0.085	0.10	0.350	0.78	0.000	0.11	0.314	0.00	1.000	0.20	0.173
b	Total	0.75	0.000	0.47	0.000	0.53	0.000	0.65	0.000	0.34	0.000	0.52	0.000
	Tree	0.85	0.000	0.53	0.000	0.42	0.000	0.80	0.000	0.49	0.000	0.58	0.000
	Tree/Shrub	0.84	0.000	0.67	0.002	0.68	0.002	0.78	0.000	0.70	0.001	0.78	0.000
	Shrub	0.60	0.005	0.50	0.015	0.75	0.001	0.42	0.031	0.22	0.142	0.56	0.008
c	Total	0.55	0.000	0.59	0.000	0.76	0.000	0.38	0.000	0.00	0.934		
	Tree	0.68	0.000	0.67	0.000	0.70	0.000	0.63	0.000	0.01	0.549		
	Tree/Shrub	0.79	0.000	0.88	0.000	0.83	0.000	0.67	0.002	0.21	0.162		
	Shrub	0.50	0.014	0.55	0.009	0.84	0.000	0.23	0.138	0.01	0.771		
d	Total	0.25	0.000			0.22	0.000	0.27	0.000	0.09	0.016		
	Tree	0.36	0.001			0.09	0.121	0.34	0.001	0.08	0.133		
	Tree/Shrub	0.40	0.038			0.02	0.714	0.52	0.013	0.19	0.180		
	Shrub	0.04	0.552			0.53	0.011	0.01	0.734	0.06	0.471		